RELATION OF THE SIZE OF "HALVES" OF THE ARBACIA PUNCTULATA EGG TO CENTRIFUGAL FORCE

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The centrifugal force used in my previous work (1932–1940) to obtain "halves"[1] of the egg of *Arbacia punctulata* has been about 10,000 × g. With this force, the egg breaks into two halves:—a (light) white half containing oil, nucleus, clear layer, mitochondrial layer and some yolk; and a (heavy) red half containing most of the yolk and all the red pigment granules. A small electric centrifuge was used, whose highest speed is approximately 10,000 R.P.M., or 160 R.P.S. The hematocrit tubes used to hold the material were 6.5 cm. long with an inside diameter of 0.35 cm. When placed in the arms of the centrifuge, the radial distance from the axis of the centrifuge to the position of the eggs while rotating was 10 cm. The centrifugal force is computed from the equation

$$F = .04 \times R \times (R.P.S.)^2$$

where R = radius in cm. and the force is expressed in times gravity.

Approximately this same force (10,000 × g) has been used by others to obtain the red and white halves for studies on permeability to water (Lucké, 1932), respiration (Shapiro, 1935), indophenol oxidase (Navez and Harvey, 1935), peptidase (Holter, 1936), and dehydrogenase (Ballentine, 1940). The segregation of different materials into the two halves has furnished a nice means of determining the location of various cell activities.

In any one batch of eggs, the white halves (and the red halves) are quite uniform in size. The *relative* size of the two halves throughout many experiments has been remarkably uniform; the white half is slightly greater in diameter than the red, and about one and one-third times greater in volume, with the force of 10,000 × g at 23° C. This I will call the standard force, since it has been so widely used.

Much greater centrifugal forces can be obtained with the air turbine.

[1] The term "halves" is used incorrectly but purposely because there is no word to express two, and only two, unequal fractions of a whole, constant in size. The term "fragments" used by some writers implies variability in size, as well as in number.

The small lucite tubes used measure 1.4 cm. in length and have an inside diameter of 0.3 cm. The radius from the center of the rotor to the position of the eggs while rotating is 1.2 cm. The highest speed obtainable with the rotor used was 1,500 R.P.S., which gives a centrifugal force of about 100,000 $\times$ g. Somewhat lower forces are obtained by using lower speeds of the air turbine; the lowest speed of the air turbine gives a slightly greater force than the highest speed of the electric centrifuge. A very low force can be obtained with a low speed of the electric centrifuge. There is then, a range in the centrifugal force available of from 4,000 $\times$ g, just sufficient to break the eggs, to 100,000 $\times$ g.

The relative size of the two half-eggs varies with the centrifugal force used to break the egg in two. As mentioned above, with the standard force of 10,000 $\times$ g, the white half is one and one-third times the volume of the red half. With a force of 60,000 $\times$ g, obtained by a

TABLE I

Arbacia punctulata. Size and force

		Diameter (μ)		Volume (μ^3)		Ratio (approx.)
Whole egg		74		212,000		
Minutes to break	Force ($\times$ g)	W	R	W	R	W : R
20	4,000	70	41	180,000	36,000	5 : 1
4	10,000	62	56	125,000	92,000	4 : 3
1	60,000	59	59	107,000	107,000	1 : 1
3/4	80,000	56	62	92,000	125,000	3 : 4
1/2	100,000	41	70	36,000	180,000	1 : 5

medium speed of the air turbine, the two halves are equal in size. With a force of 80,000 $\times$ g, the size of the two halves is the reverse of that obtained with the standard force, the red half being now one and one-third times the volume of the white. With the greatest force obtainable, 100,000 $\times$ g, the red half is five times the volume of the white. The white half is therefore very small and contains only the nucleus, some oil and a little of the clear matrix. Correspondingly, with the lowest force that will break the egg in two, 4,000 $\times$ g, obtained with a low speed of the electric centrifuge, the white half is five times the volume of the red. The greater the force, the larger the (heavy) red half and the smaller the (light) white half.

The time required to break the eggs apart at the different forces is, of course, different; it requires 20 minutes with the lowest force, and only one-half a minute with the highest force. In Table I will be found the forces used, the sizes of the two halves, their approximate ratio, and the time necessary to break approximately one-half the eggs into the two parts at 23° C. With the very high speeds of the air turbine, some inaccuracy and variability obtain, owing to the fact that the turbine must be speeded up and slowed down somewhat gradually, and the interval of full speed is so short (½ min.).

Together with the difference in size of the two halves with different forces, there is also a difference in the degree of stratification of the egg just before breaking. With a low force applied for a long period—20 minutes—the granules are entirely segregated into their respective layers according to their density, and well packed. With a somewhat higher force, the standard force, where 4–5 minutes are necessary to break the eggs apart, the granules are segregated into well-defined layers, but they are not so well packed, especially the pigment granules. With the higher forces of the air turbine, requiring only ½ to one minute, the egg breaks apart before the granules are entirely segregated so that there is no very definite stratification. Photographs 1–10 show the whole eggs centrifuged with different forces, just before breaking in two, and the two halves into which they break. With the greatest force, the materials are so poorly segregated (Photograph 5) that the red half contains some of all the materials present in the whole egg except the nucleus. By re-centrifuging the red half at the standard force, a new stratification is obtained just like that of the whole egg at this force (Photograph 12; cf. Photograph 2). A similar red half obtained with the standard force, has, when re-centrifuged with this same force, no oil or mitochondrial layer, these materials having been completely segregated out into the other half in the first centrifuging (Photograph 11).

It will also be noted that there is less elongation of the egg prior to breaking, with the higher forces.

If centrifuged slowly at first with a low speed of the electric centrifuge until well stratified, and then transferred to the air turbine and

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PLATE I

Explanation of Figures

(Magnification, 275 ×)

Photographs 1–5. Stratification of *Arbacia* eggs at different centrifugal forces, just prior to breaking in two.

Photographs 6–10. The two halves into which the eggs break at different forces.

Photograph 11. A red half obtained with 10,000 × g (Photograph 7) re-centrifuged at 10,000 × g. Control to Photograph 12.

Photograph 12. A red half obtained with 100,000 × g (Photograph 10) re-centrifuged at 10,000 × g. Note re-stratification similar to original egg at this force (Photograph 2); nucleus, of course, absent. Mitochondrial layer stained with methyl green.

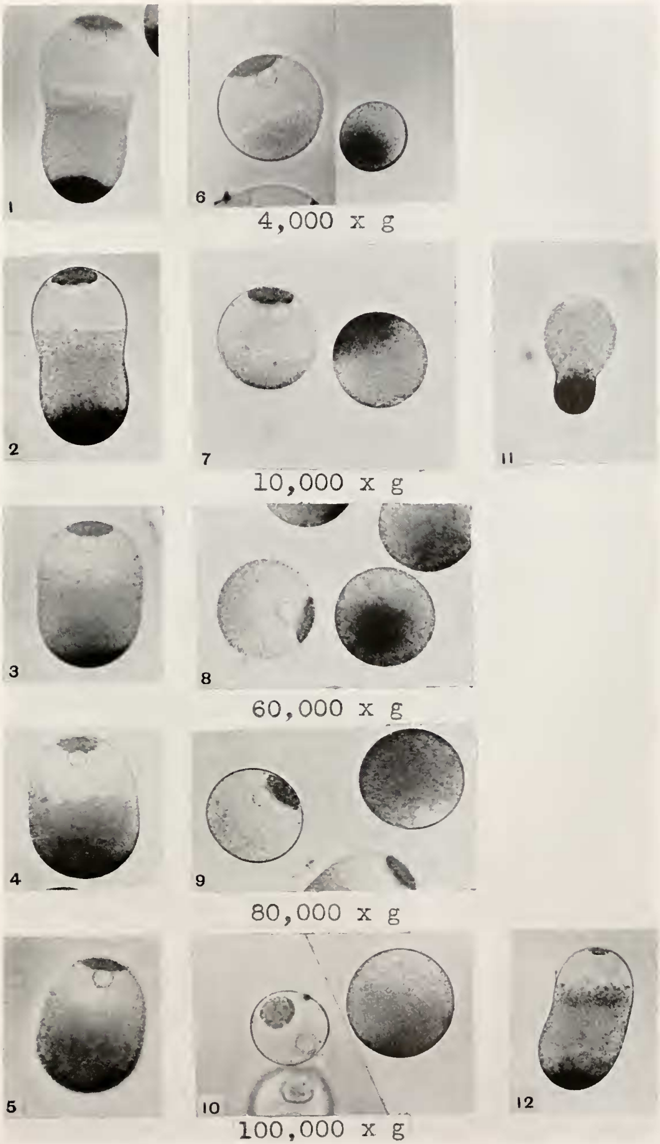
1
6
4,000 x g
2
7
11
10,000 x g
3
8
60,000 x g
4
9
80,000 x g
5
10
12
100,000 x g

PLATE I

centrifuged rapidly, the eggs break in just the same way as though they were kept at the low speed; that is, the white half is much larger than the red.

As has been noted previously (1936), several irregular batches of eggs occur each summer in which the break is quite different from the ordinary though the eggs themselves appear no different. In these eggs, at the standard force, the white halves are very large, and the red halves are very small (Photograph 16). The red halves ($D = 28.5\ \mu$; Vol. $= 12{,}000\ \mu^3$) are only about one-seventeenth the volume of the white, considerably smaller than those obtained from normal lots with the lowest force capable of breaking the egg, and even smaller than the pigment quarters obtained by breaking apart the usual red half with the standard force (Harvey, 1936, p. 103). These small red halves contain little besides the pigment granules which have been determined by E. N. Harvey (1932) to form 5.5 per cent of the materials in the egg. With a medium speed of the air turbine ($60{,}000 \times g$) the red halves of the irregular batches are much larger, but still smaller than the whites; the two halves are of about the same size as those from ordinary batches at the standard force (Photograph 17, cf. 13). With the highest speed of the air turbine ($100{,}000 \times g$), the two halves are about equal (Photograph 18, cf. 14). These irregular batches, then, show the same increase in size of the red halves with increase in centrifugal force as the normal batches, though starting from a different point.

It might be of interest to mention that these irregular batches can be made to break into the normal sized halves with the standard force, by keeping them in hypotonic sea water (80 per cent sea water, 20 per cent distilled water) for an hour and then centrifuging them in a sugar-sea water solution of the same tonicity.

The same general relationship between centrifugal force and the size of the half-eggs was found previously (1933) for the Naples sea urchins, *Arbacia pustulosa, Sphaerechinus granularis* and *Paracentrotus lividus,* within a much more limited range of forces then available. For the egg of *Parechinus microtuberculatus* (from Naples) and *Tripneustes*

PLATE II

Explanation of Figures

(Magnification, 125 ×)

Photographs 13–15. Normal eggs broken at different forces. Control to Photographs 16–18. The slightly larger sphere at the middle right in Photograph 15 is a whole egg, the others are halves.

Photographs 16–18. Irregular batch broken at same forces as 13–15.

Note uniformity in size of halves at any one force. There is usually a slight irregularity in normal batches at the highest force (Photograph 15).

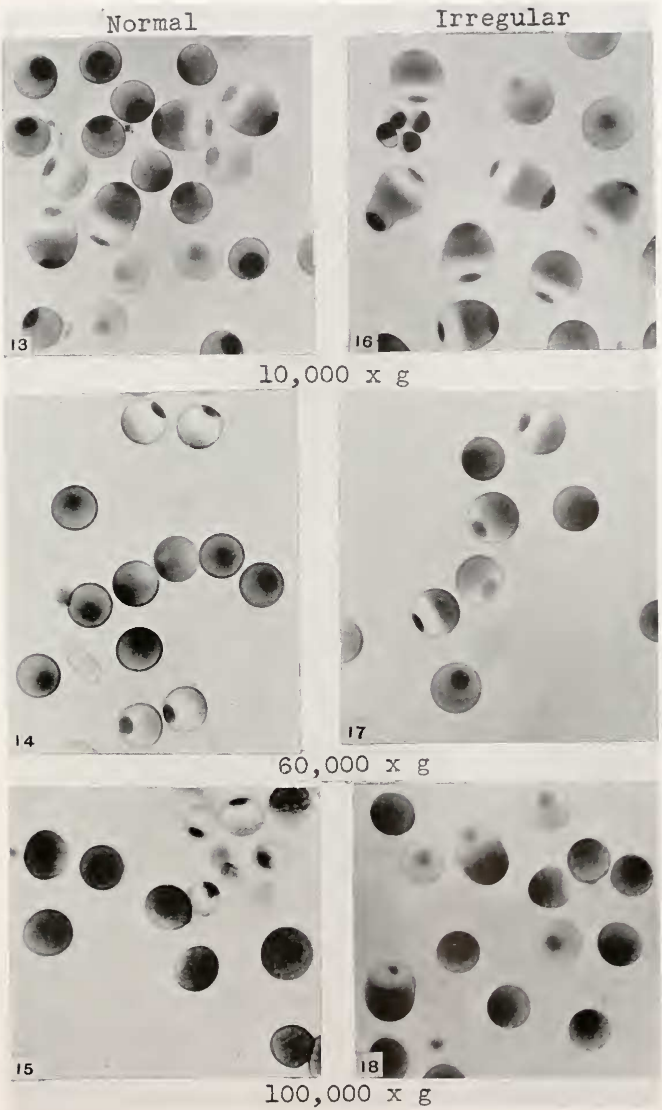

PLATE II

esculenta[2] (from Bermuda), the reverse relation was found, i.e. the greater the force, the larger the light half and the smaller the heavy half. In these eggs, the yolk granules are lighter than the matrix and lie under the oil, and the clear layer is formed more centrifugally; this must be the explanation of the apparent inconsistency in the relative size of the half-eggs of these two species. Whenever the granules are well segregated (low force) so that there is a large clear layer, the sphere with this layer is larger. Conversely, when the egg pulls apart before complete segregation of granules (high force), the clear layer is small, and the sphere containing this layer is smaller.[3]

The fact that one can vary at will the relative sizes of the two half-eggs and their composition by simply changing the centrifugal force gives a new tool for investigating the properties and functions of the different constituents of the egg. The principle may well be applied to the study of other marine eggs, and to other types of cells. For any accurate chemical work on the two half-eggs, the centrifugal force by which they are obtained must be given careful consideration. For complete segregation of materials, a low force should be used for a long period, even though one may actually break the egg in two subsequently with a high force.

Development

The small white fragments obtained with very high forces will develop when fertilized, if not too small. The smallest to develop were 32 μ in diameter, having a volume of only 7 per cent of the whole egg. Some of these formed skeletons though they did not become perfect plutei. The early cleavages of the small white fragments are quite regular.

The large red halves obtained with the high centrifugal forces cleave

[2] Data in original notes, 1932 (not published) for *Tripneustes esculenta:*

At 3,000 × g for ½ hr.	Light half, D. = 76 μ;	Vol. = 230,000 μ^3
	Heavy half, D. = 51 μ;	Vol. = 69,500 μ^3
At 7,000 × g for ¼ hr.	Light half, D. = 79 μ;	Vol. = 258,000 μ^3
	Heavy half, D. = 43 μ;	Vol. = 41,600 μ^3

The whole egg, D. = 83 μ; Vol. = 300,000 μ^3.

[3] Recent studies at Pacific Grove show that *Strongylocentrotus franciscanus* is stratified like *Parechinus* and *Tripneustes,* with the yolk granules in the light half; this half increases in size with increased force. In *Strongylocentrotus purpuratus,* two types of eggs occur, sometimes in the same batch, one type with the clear layer in the light half (like *Arbacia*), and the other type with the clear layer in the heavy half (like *S. franciscanus*). The heavy half becomes larger with increased force when it is granular, and the light half becomes larger with increased force when *it* is granular. There seems no doubt, therefore, that the distribution of the granules determines the size of the halves in relation to the centrifugal force; the more granular half becomes larger with a greater force.

more regularly and develop very much better, both fertilized and parthenogenetic, than those previously obtained with lower forces. This is surprising in a way, because one might suppose that these tremendous forces would completely disorganize the cytoplasm. It has, however, been found by Beams and King (1936) that *Ascaris suum* eggs could be centrifuged at 400,000 $\times$ g for an hour in the 2- and 4-cell stage and 90 per cent of these would still continue to develop when removed; and they would develop also after being centrifuged at 150,000 $\times$ g for 4½ days. They would even cleave while being centrifuged at 100,000 $\times$ g. These eggs are, of course, protected from disruption by a very heavy shell. The red halves of *Arbacia* are also apparently not disorganized by these high forces, and they are almost as large as the whole egg (Photograph 15) and contain some of each kind of material present in the whole egg. Only the nucleus, a little of the oil and a little of the clear matrix are lacking. This is, then, a nice technique for practically separating the nucleus from the rest of the egg. So far, these non-nucleate halves, when activated artificially—the parthenogenetic merogones—have not developed further than the blastula. If such proves finally to be the case, we may conclude that eggs can cleave and pass through the early stages of development involving cell multiplication, without nuclei, but that for the later stages involving differentiation, nuclear material is necessary.

Summary

1. *Arbacia punctulata* eggs have been broken into "halves" with centrifugal forces ranging from 4,000 $\times$ g to 100,000 $\times$ g, the higher forces being obtained with the air turbine.

2. The relative size of the two halves varies with the centrifugal force used; the higher the force the larger is the (heavy) red half, and the smaller the (light) white half. With low forces, the white half is larger than the red; with high forces, the reverse holds.

3. The degree of stratification of the eggs just prior to breaking also varies with the force used. With low forces, applied for a long period (20 min.), the eggs are very well stratified. With high forces, the egg breaks apart (½–1 min.) before the materials are completely segregated into layers.

4. The red half obtained with the highest force available (100,000 $\times$ g), is only slightly smaller than the whole egg and contains some of all the materials in the original egg except the nucleus. It develops much better, both fertilized (fertilized merogone) and parthenogenetic (parthenogenetic merogone), than the red half obtained with lower forces previously used.

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